510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k042579

B. Purpose for Submission:
New Devices

C. Measurand:
Anti- Saccharomyces Cerevisiae Antibody (ASCA), IgG and IgA

D. Type of Test:
Semi-quantitative ELISA

E. Applicant:
The Binding Site, Ltd.

F. Proprietary and Established Names:
Bindazyme™ Human ASCA IgG
Bindazyme™ Human ASCA IgA

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.5785 Anti-*Saccharomyces Cerevisiae* (ASCA) test system

2. Classification:
   II

3. Product code:
   NBT, Antibodies, *Saccharomyces Cerevisiae* (*S. Cerevisiae*)

4. Panel:
   Immunology 82

H. Intended Use:

1. Intended use(s):
   The Bindazyme Human ASCA IgG and the Bindazyme Human ASCA IgA assays are intended for the in vitro semi-quantitative measurement of ASCA IgG (Product Code MK053) or ASCA IgA (Product Code MK054), anti-Saccharomyces cerevisiae antibody, in human serum. The presence of ASCA may aid in the diagnosis of patients with Crohn's disease. The test results should be used in conjunction with clinical findings and other laboratory tests. The ASCA IgA should not be used alone as a screening test for ASCA. ASCA IgA should be used to complement, but not to replace or to substitute for the ASCA

IgG antibody test since some Crohn's disease subgroup patients may not have IgA antibodies. For *in vitro* diagnostic use only.

2. Indication(s) for use:
   The Bindazyme Human ASCA IgG assay is intended for the in vitro semi-quantitative measurement of ASCA IgG, anti-Saccharomyces cerevisiae antibody, in human serum. The presence of ASCA may aid in the diagnosis of patients with Crohn's disease. The test results should be used in conjunction with clinical findings and other laboratory tests. For *in vitro* diagnostic use only.
   The Bindazyme Human ASCA IgA assay is intended for the in vitro semi-quantitative measurement of ASCA IgA, anti-Saccharomyces cerevisiae antibody, in human serum. The presence of ASCA may aid in the diagnosis of patients with Crohn's disease. The test results should be used in conjunction with clinical findings and other laboratory tests. The ASCA IgA should not be used alone as a screening test for ASCA. ASCA IgA should be used to complement, but not to replace or to substitute for the ASCA IgG antibody test since some Crohn's disease subgroup patients may not have IgA antibodies. For *in vitro* diagnostic use only.

3. Special conditions for use statement(s):
   The devices are for prescription use only.

4. Special instrument requirements:
   Microplate reader capable of measuring OD at 450 nm.
   Microplate washing device (250-350µL repeating, or multichannel pipette, or automated system).

I. Device Description:

Each device contains the following: microplate strips with breakaway microwells coated with mannan; five levels of calibrators (100, 33.3, 11.1, 3.7, 1.23 U/mL); positive, negative and cut-off controls (diluted human serum); wash buffer concentrate; Type III sample diluent; rabbit anti-human immunoglobulin (IgG or IgA) horseradish peroxidase conjugate; 3, 3', 5, 5' Tetramethylbenzidine (TMB) substrate; 3M phosphoric acid stop solution, and Q.C. Certificate for ASCA IgG and ASCA IgA.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   INOVA QUANTA Lite™ ASCA IgG
   INOVA QUANTA Lite™ ASCA IgA

2. Predicate 510(k) number(s):
   k000732
   k000733

3. Comparison with predicate:

Similarities		
Item	New Device	Predicate Device
Intended use	To aid in the diagnosis of Crohn's disease.	Same
Technology	ELISA	Same
Assay Format	Semi-quantitative	Same
High Positive and Negative controls	Ready to use	Same
Substrate	TMB	Same
Incubation times	30-30-30 minutes	Same
Platform	96 well microtiter plates	Same
Differences		
Item	Device	Predicate
Antigen	Mannan isolated from *S.cerevisiae*	Partially purified *S.cerevisiae*
Enzyme-Conjugate	Horseradish Peroxidase rabbit anti-human IgG or IgA	Horseradish Peroxidase goat anti-human IgG or IgA
Calibrators	5 levels: 100, 33.3, 11.1, 3.7, 1.23 U/mL	None
Low positive control	None	Ready to use
Cut-off control	Ready to use	None
Sample type and dilution	Serum at 1:100	Serum at 1:101
Sample volume required	10 µL	5 µL
Sample diluent	Tris Buffered saline with Tween 20	Phosphate buffer with Tween 20. pH 7.2
Wash buffer	20x concentrate	40x concentrate
Stop solution	0.344M sulfuric acid	3M phosphoric acid
OD measurement	Within 30 minutes	Within 1 hour
OD reading	450 nm	450nm/620nm
ASCA IgG Results Interpretation	Negative: ≤ 22.0 U/mL Positive: > 22 U/mL	Negative: 0.0-20.0 Units Equivocal:20.1-24.9 Positive: ≥ 25.0 Units
ASCA IgA Results Interpretation	Negative: ≤ 7.0 U/mL Positive: > 7.0 U/mL	Negative: 0.0-20.0 Units Equivocal: 20.1-24.9 Positive: ≥ 25.0 Units

K. Standard/Guidance Document Referenced (if applicable):

None referenced.

L. Test Principle:

The Microwells are pre-coated with mannan isolated from *Saccharomyces cerevisiae*. Calibrators, controls and diluted patient samples are added to the wells and autoantibodies recognizing mannan bind during the first incubation. After washing the wells to remove all unbound proteins, peroxidase labeled rabbit anti-human IgG or IgA antibody (γ or α chain specific) conjugate is added. The conjugate binds to the captured human antibody and the excess unbound conjugate is removed by a further wash step. The bound conjugate is visualized with 3,3',5,5'tetramethylbenzidine (TMB) substrate which gives a blue reaction product, the intensity of which is proportional to the concentration of autoantibody in the sample. Phosphoric acid is added to each well to stop the reaction. This produces a yellow end point color, which is read at 450nm.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility*:

The intra-assay precision was measured using six samples, with ASCA IgG concentration level ranges from 12.1 to 67.1 U/mL, and ASCA IgA concentration level ranges from 2.9 to 67.8 U/mL. The ASCA sample ranges tested were within the two devices calibration range of 1.23 through 100 U/mL. These six samples were tested in duplicate twenty times. The % C.V. for each sample was below 10% and they are listed as follows:

INTRA-ASSAY PRECISION

ASCA IgG		
n=20	**Concentration (U/mL)**	**% C.V.**
Sample 1	12.1	3.7
Sample 2	14.2	4.2
Sample 3	33.0	4.1
Sample 4	46.8	2.3
Sample 5	67.1	6.4
Sample 6	81.3	6.7
ASCA IgA		
n=20	**Concentration (U/mL)**	**% C.V.**
Sample 1	2.9	7.3
Sample 2	3.1	4.7
Sample 3	10.5	4.1
Sample 4	15.0	2.0
Sample 5	30.9	4.7
Sample 6	67.8	2.9

The inter-assay precision was measured using eight clinical samples tested in duplicate six times for three days. The % C.V. for each sample was $\leq$12% and they are listed as follows:

INTER-ASSAY PRECISION

ASCA IgG					
n=6	**Conc (U/mL)**	**% C.V.**	**n=6**	**Conc (U/mL)**	**% C.V.**
Sample 1	10.2	8.9	Sample 5	38.4	7.8
Sample 2	12.7	10.4	Sample 6	48.7	6.6
Sample 3	28.7	7.5	Sample 7	67.9	7.9
Sample 4	37.0	8.8	Sample 8	80.0	4.7

ASCA IgA					
n=6	**Conc (U/mL)**	**% C.V.**	**n=6**	**Conc (U/mL)**	**% C.V.**
Sample 1	2.9	5.9	Sample 5	10.2	4.7
Sample 2	3.0	7.7	Sample 6	14.9	7.2
Sample 3	6.5	9.2	Sample 7	63.2	7.3
Sample 4	6.6	12.0	Sample 8	89.4	7.3

b. Linearity/assay reportable range: The measuring range of the assay is 1.23 – 100 U/mL.

i. Linearity study: Three known Crohn's positive samples with different levels of ASCA IgG or ASCA IgA were serially diluted after the initial dilution of 1:100. The values were plotted and the correlation coefficients were calculated. The ASCA IgG R^2 values on the three samples were 0.9999, 0.9985, and 0.9994, and the ASCA IgA R^2 values were 0.9991, 0.9990, and 0.9995. All samples correlate to their expected values based on the dilution and had comparable linearity.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

i. Traceability: There is no recognized standard or reference material for ASCA.

d. Detection limit: The minimum level of detection of the assay was measured the diluent 20 times. The sensitivity was based on the mean plus two standard deviations of the mean OD and was then converted to U/mL. The detection limits for ASCA IgG and ASCA IgA are 0.61 U/mL and 0.93 U/mL respectively.

e. Analytical specificity:

Interference: The substances that were tested in the interference study were Bilirubin F (Free), Bilirubin C (Conjugate), Haemoglobin, Chyle, and Rheumatoid Factor. The potentially interfering substance or corresponding blank material (water) was added at a 1/10 dilution to both a negative sample and a positive Crohn's disease sample. The samples were then assayed to determine whether the interfering substance had any effect on the assay value.

None of the above interfering substances interfered with the assay.

An additional three samples were performed on the ASCA IgA interference study, to demonstrate that results close to the cut-off of 7.0 U/mL would not be affected by the above interfering substances. Three samples ranging from 4.1 to 5.6 U/mL were tested. None of the interfering substances caused the result to change to positive. All values are within ± 20% of the sample with water added.

f. *Assay cut-off:* The cut-off value of ASCA IgG (≤ 22.0 U/mL) and ASCA IgA (≤7.0 U/mL) levels were established in serum from 200 normal blood donors. For the ASCA IgG, ten of the 200 samples (5%) were above the cut-off at 22 U/mL (approx. mean + 2SD). Using an alternative ASCA IgG kit, three of these positive samples with values of 99.2, 643.1 and 30.9 U/mL were also tested positive while the remaining 7 samples had values between 23.0 - 34.8 U/mL. This study showed that 91.5% were below the ASCA IgG cut-off. For the ASCA IgA, none of the 200 samples tested were above the cut-off at 7.0 U/mL (approx. mean + 5SD).

2. Comparison studies:

a. *Method comparison with predicate device:*
Comparison was determined against the predicate ASCA IgG and IgA EIA kits using 120 normal and 105 samples from patients with clinically confirmed Crohn's disease. Samples in the equivocal range in alternative kits (4 samples) were excluded from the analysis. Results are summarized below.

		QUANTA Lite™ ASCA IgG		
		+	Equivocal*	-
BINDAZYME ASCA IgG	+	47	0	5
	-	20	4	149
Total		67	4	154
Positive Percent Agreement		70.2%		
Negative Percent Agreement		96.8%		
Overall Agreement		88.7%		

		QUANTA Lite™ ASCA IgA		
		+	Equivocal*	-
BINDAZYME ASCA IgA	+	32	3	8
	-	3	1	178
Total		35	4	186
Positive Percent Agreement		91.4%		
Negative Percent Agreement		95.7%		
Overall Agreement		95.0%		

b. Matrix comparison:
Both assays use serum as the matrix.

3. Clinical studies:
   a. Clinical sensitivity:
   Study on other GI Inflammatory Disease Groups*:* One hundred and five samples from clinically confirmed Crohn's disease (CD) and 96 clinically confirmed ulcerative colitis (UC) patients were tested. Of the 31 samples positive for both ASCA IgG and IgA, 27 (87%) were from the Crohn's Disease group. The incidence of positive results in the normal sample group supports the reported incidence in the normal range study of the Bindazyme ASCA EIA kits (refer to the Assay Cut-off section). The table below shows the results:

BINDAZYME™ ASCA EIA Result					
Group	**N**	**IgG Pos (n)**	**IgA Pos (n)**	**IgG or IgA positive (n)**	**IgG and IgA Pos (n)**
Crohn's Disease	105	44% (46)	37% (39)	55% (58)	26% (27)
UC	96	14% (13)	6% (6)	17% (16)	3% (3)
Normals	120	5% (6)	3% (4)	8% (9)	1% (1)

   b. Clinical specificity:
   ASCA Study on Celiac and ANA positive samples: Study results on the incidence of ASCA in samples from patients positive for antibody markers of celiac disease and samples positive for antinuclear antibodies are listed in the table below:

Sample	N	ASCA IgG pos	ASCA IgA pos
Gliadin IgG pos	11	2	3
Gliadin IgA pos	14	6	2
Transglutaminase IgA pos	9	3	2
Transglutaminase IgA pos	4	0	0
Antinuclear antibody pos	10	0	0
GBM positive	10	2	1

   c. Other clinical supportive data (when a. and b. are not applicable):
   Not applicable.

4. Clinical cut-off**:**
   Same as assay cut-off.

5. Expected values/Reference range:
   Expected values in the normal population using the Bindazyme ASCA assays are ≤22.0 U/mL for ASCA IgG and ≤7.0 U/mL for ASCA IgA.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.